gland on the right side of the intestine, and that the stalks of water weeds and other objects of which the nest is constructed, are bound together by compound threads of six or eight fibers spun by him in a fitful way as the material is secreted.

The egg-membranes of floating fish ova, as those of *Cybium maculatum*, are extremely thin, and pierced only by the micropyle, not perforated by pore canals as is the case with ova, which like those of the stickleback, salmon and shad, sink to the bottom. The ova of *C. maculatum*, the Spanish mackerel, are hatched in twenty-four hours after fertilization, and the young are then in a very rudimentary state.

The gills of the so-called Lophobranchiates are not really tufted, but the two series of vascular branchial appendages to each arch in Hippocampus are homologous to the bifurcated vascular branchial appendages of a salmon or other fish. But these appendages are much reduced in number, and, as if to compensate for this, the area of the ultimate branchial lamellæ or pinnæ ranged upon them is extended, and these leaflets increase in size outwards, producing a tufted appearance. In all Lophobranchs the branchial arches are reduced, the opercle is a simple plate, the mouth is toothless, and the opercular membrane persistently roofs over the gill chambers of the embryos.

Experiments upon the retardation of the development of the ova of the shad, with the end of ascertaining the possibility of transporting them alive for long distances, were not successful on account of the development of fungus, but in four and a half days the ova at a temperature of 52° F., had not advanced farther than they would have done in water at 80° in twenty-four hours.

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METHODS OF MICROSCOPICAL RESEARCH IN THE ZOOLOGICAL STATION IN NAPLES.

BY C. O. WHITMAN.

(Continued from September number.)

II. Staining Methods.

IT has gradually become a settled custom in the Zoölogical Station, to mount microscopical preparations in balsam wherever this can be successfully done; and to avoid, as much as possible, the use of aqueous media, both in mounting and staining. The disadvantages often arising from the use of these media in stain-

ing alcoholic preparations, such as the tearing asunder of fragile tissues caused by the violent osmosis; swelling, the effects of which cannot always be fully obliterated by again transferring to alcohol, and maceration, which is liable to result where objects are left for a considerable time in the staining liquid, may all be avoided by using alcoholic solutions. Objects once successfully hardened may be left in such solutions for any required time, and when sufficiently stained, be washed in alcohol of a corresponding strength, and then passed through the higher grades without being exposed to water from first to last. As a rule, alcoholic dyes work quickly, and give far more satisfactory results than can be obtained with other media. They penetrate objects more readily, and thus give a more uniform coloring where objects are immersed in toto. Even chitinous envelopes are seldom able to prevent the action of these fluids.

It is not, however, to be denied that non-alcoholic dyes may often do excellent work, and in certain cases, even better than can be otherwise obtained. In the case of the Turbellaria, Dr. Lang has found picro-carmine to be one of the best staining agents, and this has been my experience with Dicyemidæ. As Dr. Mayer has remarked, the swelling caused by aqueous staining fluids is not always an evil, but precisely what is required by some objects after particular methods of treatment.

From experiments recently made, Dr. Mayer has found that dyes containing a high percentage of alcohol, stain more diffusely than those of weaker grades, from which he infers that strong alcohol robs, to a certain extent, the tissues of their selective power, and renders them more or less equally receptive of coloring matter.

1. *Kleinenberg's Hæmatoxylin.*[1]—1. To a saturated solution of chloride of calcium[2] in 70 per cent. alcohol, add a little alum and filter.

2. One volume of No. 1 mixed with six to eight volumes of 70 per cent. alcohol.

3. At time of using pour into No. 2 as many drops of a con-

[1] May be used after all hardening fluids.

[2] Chloride of calcium, according to Kleinenberg, has no other use than to strengthen the osmotic action between the hæmatoxylin solution and the alcohol contained in the tissues. As chloride of calcium and alum give a precipitate of gypsum, it would probably be better to use *chloride of aluminum.*

centrated solution of crystallized hæmatoxylin in absolute alcohol as suffice to give the required depth of color.[1]

If the color appears too strong, the fluid may be diluted with solution No. 1.

Before immersing objects in this fluid, great care should be taken to free them from the least trace of acid by frequently changing the alcohol. If this is not done thoroughly, the acid left in the preparation will sooner or later cause the color to fade; and such results have led to the erroneous conclusion that hæmatoxylin will not give durable preparations. Dr. Mayer has found that the fading is entirely due to the presence of acid, and that with proper precautions the staining is permanent.

Small objects are best stained in a weak solution, which colors more slowly but with greater clearness than stronger solutions. After staining, Kleinenberg transfers objects to 90 per cent. alcohol. In case of over staining, the color may be partly removed by adding a little *oxalic acid* or *hydrochloric acid* (½ per cent. or less) to the alcohol containing the objects. The acidulated alcohol is allowed to work until the color is slightly reddened. On transferring to pure alcohol the color passes again into a permanent blue-violet.

2. *Mayer's cochineal tincture.*—1 gramme powdered cochineal soaked in 8–10 ccm. 70 per cent. alcohol for several days, then filtered.

The clear deep red fluid thus prepared may, like hæmatoxylin, be used in all cases where it is desirable to stain with an alcoholic solution, and will be found particularly useful for objects that are not easily penetrated by the ordinary aqueous solutions of carmine, such as the Arthropods.

It is necessary, before immersing larger objects in this fluid, to leave them a short time in 70 per cent. alcohol, otherwise there may be a precipitate. The time required for staining, will vary from a few minutes to even days, according to the nature and size of the object. With larger objects requiring considerable time,

[1] A good solution should be violet inclining a little to blue. The red tinge that arises after the fluid has stood for some time, indicates that it has become slightly acid, in which condition it is unfit for use. To restore its proper color, it is only necessary to open a bottle of ammonia over the mouth of the bottle holding the hæmatoxylin in such a manner that a very small quantity of the gas will mix with the fluid. If too much ammonia gas be added, a precipitate is produced which spoils the fluid.

it is important to use a large quantity of the fluid, otherwise the amount of coloring stuff in solution might not suffice to give the proper depth of color. Small and delicate objects, on the other hand, may be most successfully treated with a solution which has been diluted with 70 per cent. alcohol, or one which has been weakened by previous use. It is always necessary to free the tissues, after staining, from the surplus dye; and this may be done by washing in 70 per cent. alcohol, which must be changed until it shows no color. This process requires, for larger objects, considerable time and alcohol, but may be hastened by using the alcohol slightly warm.

The color ultimately assumed by objects treated with cochineal tincture varies much, and depends partly on the reaction of the tissues themselves, partly on the presence or absence of certain salts. It is certainly one of the best recommendations of this staining agent that, varying with the nature of the object and its mode of treatment both before and after staining, it gives such an extraordinary diversity of results. On account of the great variety of substances contained in the dried dye-stuff, it is evident that the composition of the tincture must vary according to the strength of the alcohol employed as a solvent. Solutions in 90 per. cent. or 100 per cent. alcohol have a light red color, and stain too diffusely to have any practical value. The weaker the alcohol the stronger the tincture, and the stronger the alcohol the more easily it penetrates objects; the grade of alcohol may therefore be selected with reference to two points, depth of color and readiness of penetration; 70 per cent. or 60 per cent. is recommended by Dr. Mayer as combining both these qualities in a very favorable degree. It is important to remember that whatever be the strength of the solution, a precipitate will always be produced if an alcohol of a different grade, whether higher or lower, be mixed with it. It is evident then that a tincture of any given strength contains substances that are insoluble in any other grade of alcohol, and this explains why superfluous coloring matter can only be removed from objects by the aid of alcohol of precisely the same degree as that of the tincture.

Over staining, which seldom occurs, may be easily corrected by the aid of acid alcohol ($\frac{1}{10}$ per cent. hydrochloric acid, or 1 per cent. acetic acid). Acid makes the tincture lighter, more yellowish-red, while the addition of ammonia and other caustic alkalies

changes it to deep purple. Still more important is the fact that salts soluble in alcohol give a blue-gray, green-gray or blue-black precipitate. For example, if a piece of cloth that has been dyed in cochineal and washed, be treated with an alcoholic solution of a ferric or a calcic salt, it will assume a more or less deep blue color.

As the salts present in the living organism are seldom, if ever, fully removed by preservative fluids, but in some cases even increased, it will often happen that an object, though stained in the red fluid, comes out blue, precisely as when stained with hæmatoxylin. Such a result cannot, however, be obtained in the presence of acids, nor in the absence of inorganic salts; under these conditions the color is always red. It is not possible, therefore, to know what color an object will ultimately present.

Very often the different tissues of one and the same object present unlike colors. In the embryos of Lumbricus, Kleinenberg found the walls of the blood vessels red, their contents dark blue. Glandular tissues, or their contents, are frequently stained gray-green.

Objects treated with chromic or picric solutions, or with alcohol, usually stain without difficulty; but osmic acid preparations should be bleached before staining. Cochineal does not color so intensely as hæmatoxylin, and hence the latter often gives more satisfactory results in the case of large objects stained in toto.

As before pointed out, alcohol causes the salts contained in sea water to be precipitated, thus forming a crust on the exterior of the animal which interferes with the staining process. It is therefore necessary to treat marine animals that have been preserved in strong alcohol, with acid alcohol (1–10 parts hydrochloric acid to 1000 parts 70 per cent. alcohol), and then carefully wash in pure 70 per cent. alcohol before staining with cochineal.

3. Picro-carmine.—A very excellent picro-carmine is prepared by Dr. Mayer in the following manner:

To a mixture of powdered carmine (2 g.) with water (25 ccm.), while heating over a water bath, add sufficient ammonia to dissolve the carmine. The solution may then be left open for a few weeks (Mayer) in order that the ammonia may evaporate; or the evaporation may be accelerated by heating (Hoyer). So long as any ammonia remains, large bubbles will form while boiling, but as soon as the free ammonia has been expelled, the bubbles will

be small and the color of the fluid begin to be a little lighter. It is then allowed to cool, and filtered. To the filtered solution is added a concentrated aqueous solution of picric acid (about four volumes of the acid to one of the carmine solution).[1]

In order to protect this fluid against changes attributed to Bacteria by Hoyer,[2] Dr. Mayer places a small crystal of *thymol* in the containing bottle; Professor Hoyer uses *chloral-hydrate* (1 per cent. or more) for the same purpose.

4. Acetic Acid Carmine.[3]—Pulverized carmine added to a small quantity of boiling acetic acid (45 per cent.) until no more will dissolve; filtered and diluted to about 1 per cent. for use.

Flemming used the concentrated solution.

5. Grenacher's Carmine Solutions.[4]—(1) *Alum Carmine.*—An aqueous solution of alum (1–5 per cent., or any degree of concentration) boiled with ½–1 per cent. powdered carmine for 10–20 minutes; allowed to cool, then filtered.

With the addition of a little carbolic acid the fluid will keep for years. It colors quickly, and nuclei more strongly than other parts. Objects washed in water after staining.

(2) *Acid Borax Carmine.*—*a.* An aqueous solution of *borax* (1–2 per cent.) and *carmine* (½–¾ per cent.) heated till the carmine is dissolved.

b. Acetic acid added by drops to solution *a*, while shaking, until the color is about the same as that of Beale's carmine.

c. Solution *b* left standing twenty-four hours, then turned off and filtered.

This solution, which is a modification of Schweigger-Seidel's acid carmine, is not recommended for coloring in toto. It colors sections in ½–3 minutes diffusely, and hence, after washing in water, they are placed for a few minutes in alcohol (50 or 70 per cent.) to which a drop of hydrochloric acid has been added; then transferred to pure alcohol.

[1] The addition of the acid should cease before a precipitate begins to form.

[2] Hoyer. "Beiträge z. histolog. Technik." In Biolog. Centralblatt, B. II, p. 17–19.

[3] Schneider. Zool. Anzeiger, No. 56, p. 254, 1880.

[4] Grenacher. "Einige Notizen z. Tinctionstechnik." Arch. f. Mik. Anat., Vol. XVI, p. 463, 1879.

None of these solutions to be used where calcareous parts are to be preserved.

(3) *Borax Carmine.*[1]—*a.* An aqueous solution of *borax* (4 per cent.) and *carmine*, heated till the carmine is dissolved.

b. Solution *a* mixed with 70 per cent. alcohol in equal parts, left standing twenty-four hours and filtered.

This fluid may be used for coloring objects in toto. After staining, the objects are to be washed in 35 per cent. alcohol, to which a little hydrochloric acid has been added (4–6 drops to 100 ccm.), and allowed to remain here until the color has been sufficiently removed. They are next passed through successively higher grades of alcohol for hardening.

(4) *Alcohol Carmine.*—A teaspoonful of carmine dissolved, by heating about ten minutes, in 50 ccm. of 60–80 per cent. alcohol, to which 3–4 drops of hydrochloric acid have been added, then filtered.

Objects colored in this fluid should not be washed in water, but in alcohol of a grade corresponding to that of the solution.

For diluting alcoholic solutions of carmine, alcohol of the same strength must always be used.

6. Aniline Dyes.—As a rule, aniline colors and the many others obtained recently from tar by chemical processes, can not be used for staining objects in toto, and are therefore not much employed in the Zoölogical Station. In very small objects and sections already cut, very excellent results can be obtained by the methods developed by Böttcher,[2] Hermann,[3] Flemming[4] and others; for here diffuse staining may generally be avoided by first overstaining and then withdrawing the color to any desired extent by means of alcohol. But to obtain satisfactory results, the sections must be thin enough to allow uniformity of action both to the coloring and the decoloring agent. It is evident that the process cannot be similarly controlled in larger objects, particularly where a dye is used, which, like most of those under consideration, is quickly extracted by alcohol, for in this case the color would be removed from the superficial layers more rapidly than from the deeper

[1] Dr. Mayer prepares, for some purposes, borax carmine of 50, 60 or 70 per cent. That of 70 per cent. contains little carmine, but is well adapted to staining delicate objects that would suffer if exposed to weaker solutions. Boiling alcohol (50 per cent. or 60 per cent.) dissolves about 1 per cent carmine and 1 per cent. borax.

[2] Bottcher. *Mul. Archiv.*, 1869, p. 373. Virchow's Archiv., Bd. XL, p. 302.

[3] Hermann. Communicated to the Naturforscherversammlung in Graz, 1875. Tagblatt, p.105,

[4] Flemming. Archiv. f. Mikr. Anat., Bd. XIII, p. 702, Bd. XVI, p. 302, Bd. XVIII, p. 151, Bd. XIX, p. 317, and p. 742, B. XX, p. 1.

ones, so that a uniform precision of color would be impossible. In this respect,

a. Bismarck-brown forms an exception. The preparation of this dye, introduced by Weigert,[1] is extremely simple:

A saturated solution is made by dissolving the powder in boiling water or weak alcohol, or, according to Mayer, in 70 per cent. alcohol.[2] The solution should be used undiluted, and requires to be filtered from time to time. It colors very quickly objects hardened in alcohol or chromic acid.

b. Safranin.—1 part *safranin* dissolved in 100 parts of *absolute alcohol;* after a few days 200 parts of *distilled water* is added.

Dr. Pfitzner,[3] from whom the above formula is taken, recommends this solution as one of the best for staining nuclei. It is cheap, easily prepared, acts quickly and stains *only* the nuclei. It works best with chromic acid preparations, from which the acid has been removed as much as possible.

7. Flemming's methods of treating Nuclei.—The method employed by Böttcher and Hermann of *over staining* objects with aniline dyes, and then removing the color to any desired extent by the aid of alcohol, formed the starting point of the methods recently published by Flemming. The following is a summary of the more important conclusions reached by Flemming:[4]

A. For Nuclei in general.—1. Objects hardened in *chromic acid* (1-10 per cent. to ½ per cent.).

The time will vary according to the nature of the object.

2. Carefully washed in distilled water.

3. Stained directly, or further hardened in weak and then strong alcohol.

Safranin, Magdala red (rose de naphthaline) and *dahlia* (monophenylrosanilin) give the best staining. Safranin prepared as given above; magdala in the same way; dahlia best dissolved in water, or acetic acid.

Only very small objects, or thin sections, can be successfully stained, and these should be left in the fluid 12–24 hours.

4. Objects transferred to weak alcohol (70 per cent.) and shaken for a few moments; then placed in absolute alcohol for half a minute or longer—till no visible clouds of color appear. The process of decoloring is now completed and the objects must be

[1] Wiegert. Arch. f. Mik. Anat., Bd. XV, p. 258, 1878.

[2] According to Flemming, may also be dissolved in dilute acetic acid.

[3] Pfitzner. Morph. Jahrb., VI, pp. 478–80 and VII, p. 291.

[4] Flemming. Archiv. f. Mik. Anat., Vol. XIX, p. 321.

at once removed from the alcohol, otherwise the color will be too much weakened. If it be required to examine the objects before mounting, they may be removed to distilled water, in which the color of the nuclei will remain unchanged for a considerable time. They must then pass through alcohol again before mounting.

5. Clarified in clove oil and mounted in *dammar-lac*.[1]

Clove oil withdraws the color a little, and hence it must not be allowed to work too long. Creosote extracts the color still more rapidly than clove oil.

B. Eggs of Echinoderms.[2]—In his recent researches on karyokinesis, Flemming states (p. 5) that he obtained serviceable staining of nuclei in the following ways:

1. *Living eggs colored on the slide*, either with *safranin* or *aniline dyes*, followed by acetic acid (1 per cent.) which is allowed to flow under the cover and thus replace the staining medium, or with

Acetic acid carmine (after Schneider), used undiluted. The last mentioned staining agent causes swelling, but still gives the typical features of the karyokinetic figures.

2. Eggs first hardened in strong nitric acid (40–50 to aq. dest. 60–50), then washed in distilled water until the yellowish color, due to the presence of the acid, disappears. Colored with acetic acid carmine.

III. Methods of Dissecting.

For the dissection of single organs, fresh animals are generally placed in dilute alcohol, or a weak chromic solution. But the tissues are liable to suffer from maceration in these fluids, and hence, where it is important that the tissues should be well preserved, it is advisable to use picro-sulphuric acid, regardless of the injurious effects of the same on the dissecting instruments. The hardening capacity of the picro-sulphuric acid is extremely slight, but may be strengthened by the addition of chromic acid. Preparations thus obtained, and subsequently treated with alcohol, staining fluids, &c., should be transferred to creosote for further dissection, as the transparency induced by this medium will greatly facilitate the work.

IV. Imbedding.

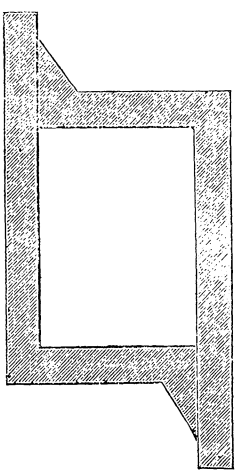
For section cutting, objects are usually imbedded in paraffine. By low temperature, as in winter, it is necessary to work with a softer paraffine than is required for summer. Instead of soften-

[1] Probably balsam dissolved in chloroform would answer the same purpose.

[2] Flemming. "Beitrage zur Kenntniss der Zelle und ihrer Lebenserscheinungen." Arch. Mik. Anat., Vol. xx, p. 1, 1881.

ing by an admixture of lard, as generally done, it is better to use a paraffine which becomes soft in summer, on account of its containing liquid hydrocarbons.

Preparatory to imbedding, the objects are removed from absolute alcohol[1] to creosote, clove oil or chloroform, and left until they become thoroughly saturated. The penetration of the clarifying fluid may, in some cases, be advantageously hastened by warming a little. They are next placed in soft paraffine, heated to about 50° C. over a water bath, and allowed to remain for an hour or so. The soft paraffine is then turned off and replaced by a mixture of hard and soft paraffine,[2] heated to about 50° C. After remaining for half an hour or less in the harder paraffine, kept at a steady temperature, they are ready for imbedding. For this purpose a small paper box may be used; or, much better, a box made of two pieces of type-metal, as used in Professor Leuckart's laboratory. As will be seen from the accompanying diagram, each piece of metal has the form of a carpenter's square, with the end of the shorter arm triangularly enlarged outward. A convenient size will be found in pieces measuring 7 (long arm) by 3^{cm} (short arm), and 7^{mm} high. With such pieces a box may be constructed at any moment by simply placing them together on a round plate of glass, which has previously been wet with glycerine and gently warmed. The area of the box will evidently vary according to the position given to the pieces, but the height can be varied only by using different sets of pieces. In such a box the paraffine may be kept in a liquid state by warming now and then over a spirit lamp, and small objects be placed in any desired position under the microscope.



It is well to imbed in a thin layer of paraffine, so that the object, after cooling, may be cut out in small cubical blocks, which

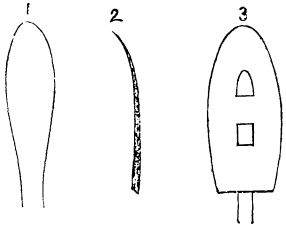
[1] In many cases a lower grade of alcohol will suffice.

[2] The ratio of combination must be determined by experiment, since it will depend on the quality of the paraffine and the temperature. Two parts of hard to one of soft, work very well for the winter temperature of Naples.

may be easily fixed, for cutting, to a larger block of hard paraffine.

V. Cutting.

Objects are cut dry with a microtome,[1] and the rolling of the sections may be prevented by holding a thin narrow spatula over the edge of the knife while cutting. The spatula may be made of brass, in the form of Fig. 1; of paper fastened to a flattened needle as indicated in Fig. 3. The spatula should be bent slightly (Fig. 2), and its convex face held over the paraffine without pressure. A small brush, slightly flattened, is used for the same purpose in Leipsic.



VI. Giesbrecht's Methods.

(1) *Transferring from Alcohol to a solvent of Paraffine.*[2]—To avoid shrinkage in transferring tender objects from alcohol to chloroform or an oil, pour a little absolute alcohol into a small glass tube, place the canular end of a pipette containing the solvent below the surface of the alcohol, and allow a few drops to flow from it to the bottom of the tube; into this tube let fall, by the aid of another pipette, or a small spatula, a few drops of absolute alcohol containing the objects to be imbedded. The objects will sink through the alcohol, which, being the lighter fluid, has taken a superjacent position, and rest on the upper surface of the fluid expelled from the first pipette. Most of the alcohol may now be removed by a pipette, and the objects left to sink gradually into the heavier fluid at the bottom of the tube. In this way the replacement of the alcohol contained in the objects by an oil, or some solvent of paraffine, is much retarded, and thus the danger from shrinkage reduced to a minimum.

Where chloroform is preferred to creosote or oil of cloves, a little ether (æther sulfuricus C_4H_5O) should be added, as many objects will not sink in pure chloroform.

To replace alcohol by a solvent of paraffine, and then by par-

[1] An improved form of Thoma's microtome is made by Rudolph Yung, Heidelberg, Hauptstrasse 15. The carrier is moved by a micrometer screw, and the holder can be adjusted in any desired position. A full description of the instrument with all the recent improvements will soon be given by Dr. Mayer.

[2] Giesbrecht. "Zur Schneide-Technik," in Zoolog. Anzeiger, 1881, No. 92.

affine itself, is an operation which may, in many cases, be readily accomplished by employing any one of the ordinary intermedia, such as oil of cloves, bergamot oil, creosote, turpentine, chloroform, &c. But with tender objects, particularly those with larger or smaller internal cavities, the process is often attended with great difficulties, and in such cases collapse and shriveling can only be avoided by giving the most careful attention to every step in the process.

Dr. Giesbrecht recommends, for difficult cases, chloroform,[1] as it is one of the best, and at the same time the most volatile solvent of paraffine.

(2) *Transferring from Chloroform to Paraffine.* — After the objects have become thoroughly saturated with chloroform, the containing tube is placed on a water bath and heated to about 50° C.—the melting point of paraffine; then a small piece of paraffine is added and allowed to dissolve, and this is repeated until bubbles cease to rise from the objects. To make sure that the chloroform has been fully expelled, the objects may next be transferred to pure paraffine and left for a few minutes before imbedding.[2]

(3) *Shellac as an aid in Mounting.*—The use of *shellac* for fixing sections on the slide, introduced by Dr. Giesbrecht,[3] is a very valuable addition to histological methods. By this method hundreds of small sections may be arranged in serial order, and all inclosed in balsam under the same cover without danger of disarrangement. The method is further extremely useful in mounting larger sections, particularly those composed of loose parts, or parts liable to swim apart.

[1] Bütschli (Biolog. Centralblatt, B. 1, p. 591) has also recommended chloroform, entirely overlooking, as it would seem, Dr. Giesbrecht's prior publication.

[2] For the Hydrozoa, Professor Weismann prefers turpentine to chloroform, as where the latter has been used, the paraffine is liable to be more or less spongy in consequence of bubbles lodged in the tissues.

Turpentine renders objects brittle, and on this account chloroform will, in many cases give better results. The spongy state of the paraffine results from the fact that the chloroform has not been allowed to wholly escape.

In the case of the Actiniæ, Dr. Andres employs a mixture of turpentine, creosote and alcohol, using successively mixtures containing more turpentine and less alcohol, thus:

Mixture No. 1.	No. 2.	No. 3.	No. 4.
Turpentine..... 1	2½	4½	7½ parts.
Creosote..... 2	2½	2½	2½ "
Alcohol (absolute)..... 7	5	3	0 "

[3] Giesbrecht. "Methode zur Anfertigung von Serien-Praparaten," in Mittheilungen a. d. Zoolog. Station zu Neapel, 1881, p. 184.

The shellac is prepared and used in the following manner: One part of bleached shellac[1] mixed with ten parts absolute alcohol, and filtered. The object glass is first warmed to about 50° C.,[2] and then a thin film of the shellac is laid on by a glass rod drawn once over its surface. Before using, the slide is again warmed, and the shellac surface washed with oil of cloves for the purpose of softening it. The wash is made with a small brush drawn back and forth until the entire surface has been moderately but evenly wet with the oil. Sections are now cut and arranged for the first cover; this done, the slide is warmed over a spirit lamp so that the paraffine adhering to the sections melts and flows together, forming an even layer which cools almost instantly, and thus secures the position of the sections while those of the second cover are prepared. The sections for the last cover having been completed, the slide is warmed for ten minutes on a water bath, in order that the sections may sink into the shellac and become fixed, and the clove oil evaporate. After allowing the slide to cool the process is concluded by washing away the paraffine with turpentine, and inclosing in balsam dissolved in chloroform.[3]

[1] Dr. Mark informs me that he uses "the bleached shellac in the form in which it is prepared for artists as a '*fixative*' for charcoal pictures. It is perfectly transparent, and a film of it cannot be detected unless the surface is scratched." Dr. Mark attaches a small label to the corner of the slide, which serves for the number of the slide and the order of the sections, and at the same time marks the shellac side (otherwise not distinguishable).

[2] The same temperature is used throughout the operation.

[3] Since the above was written, my attention has been called to the following mode of fixing sections, first described by Dr. Gaule (Archiv. f. Anat. u. Phys., 1881, Phys. Abthlg., p. 156):

1. Sections cut dry and placed on the slide in the order and position in which they are to be mounted.

2. They are then smoothed out by the aid of a fine brush wet in 50–60 per cent. alcohol, until all wrinkles are removed and every part is in close contact with the slide.

3. Slide allowed to stand several hours (or over night) until the alcohol has completely evaporated, and the sections are left adhering quite firmly to the glass. The process may be hastened by gently warming to 45–50° C.

4. The paraffine may be removed by any of the solvents in common use, but Dr. Gaule recommends Xylol. A few drops are allowed to flow over the sections, and after a few moments the paraffine is fully dissolved.

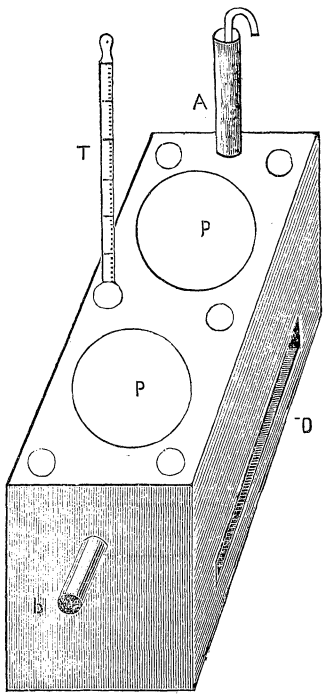
5. The balsam (a mixture of balsam and xylol in equal parts) is placed on the cover-glass, and this allowed to sink slowly, from one side, over the sections.

Dr. Gaule finds it convenient, especially with serial sections, to use large cover-glasses—often nearly as large as the slide itself. Thus a single slide may often contain a large number of sections closely arranged under one cover.

For large sections this method offers one important advantage over that of Dr. Giesbrecht; for by the former all wrinkles may be removed, while by the latter the sections must lie as they fall. In the case of smaller sections, not liable to get wrinkled during the placing, I prefer the shellac method.

WATER BATH.

The diagram represents a convenient form of water bath, devised by Dr. Mayer.



It is a small brass box 18^{cm} long, 9^{cm} wide and 8^{cm} high. The tube *a*, through which the water is received, and the rod *b* serve as handles. The receiving tube is closed by a cork provided with a glass tube for the escape of steam, which is bent in the form of a siphon to protect against dust. One and a-half centimeters from the base of the box is an oven (*o*) .7^{cm} high, and 12^{cm} long, which passes completely through the box, and serves for warming the slides when shellac is used. Above are seen two circular basin-like pits (*p*) 5.5^{cm} in diam., and 4^{cm} deep, for receiving the two tin paraffine holders. These are covered by circular plates of glass. There are also six tubular pits, one for a thermometer (*t*), the others for glass tubes.

This water bath will be found useful for other purposes than those of imbedding and mounting. It will of course be understood that the purpose in giving its exact dimensions is simply to furnish a guide where one is required. There are at least two important advantages offered by this water bath over those in general use, viz., the slides are protected from dust, and the paraffine is not exposed to the water.

——:o:——

ON THE HOMOLOGIES OF THE CRUSTACEAN LIMB.

BY A. S. PACKARD, JR.

THE following observations are reprinted from an essay on North American Phyllopod Crustacea, contributed to the forthcoming Twelfth Annual Report of the U. S. Geological and Geographical Survey of the Territories, F. V. Hayden in charge. I am indebted to Dr. Hayden's kindness for the use of the illus-